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TOXICOKINETICS METABOLISM AND GENOTOXICITY OF NITROPROPANE IN RATS AND MICE

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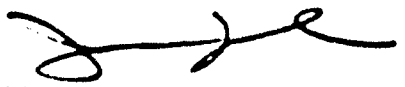
The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER



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13. ABSTRACT (Maximum 200 words) Pharmacokinetics of atmospheric 2-nitropropane (2-NP) were investigated in female and male rats at initial concentrations between 100 and 3000 ppm using closed exposure systems. A thermodynamic partition coefficient (body/air) of 180 was obtained from experiments in vivo. A value of 161 was estimated from experiments in-vitro. Due to the metabolism in the animals, the actual concentration in steady state (body/air) was far below these values. At concentrations below 10 ppm it was 23 and 30 in male and female rats, respectively. 2-NP was found to be metabolized via two pathways, a non-saturable one according to first-order kinetics which was quite similar in both sexes, and a saturable one according to Michealis-Menten kinetics, females having a higher metabolic capacity than males. The share of the non-saturable pathway was therefore higher in males than in females and exceeded the share of the saturable pathway above 60 ppm in males and above 180 ppm in females. IP administration of 2-NP (1.7 mmol/kg b.w.) to rats resulted in an acute hepatotoxicity indicated by an increase of liver enzymes (GOT, GPT, and OCT) in serum with a peak concentration after 8 h. The values in males were much higher than in females and showed a dose response curve at concentrations from 0.13 to 3.4 mmol/kg of 2-NP. Exposure of rats, 4-5 days old, to 2-NP (0 - 125 ppm) for 3 weeks and subsequent treatment with Clophen A50 resulted in the formation of ATPase-deficient preneoplastic foci. The dose dependency resembled that of the nonsaturable (Cont'd)

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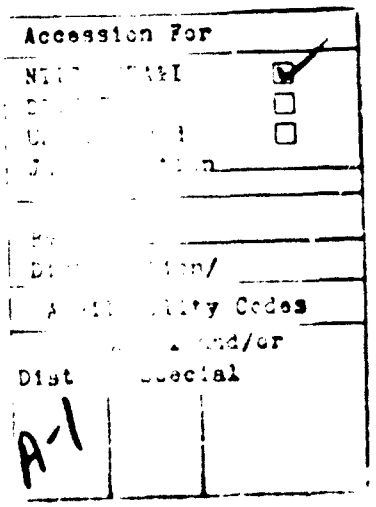
pathway. A program "SOLVEKIN" was developed which is able to solve special toxicokinetic problems as simulation and parameter estimation. The program will be used for pharmacokinetic modelling.



TOXICOKINETICS, METABOLISM, AND GENOTOXICITY OF NITROPROPANES IN RATS AND MICE

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INTERIM SCIENTIFIC REPORT

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Author: PD Dr. Johannes G. Fiser

Study assistants: Dr. Winfried Kessler
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GENERAL

Until now, following issues concerning the project have been investigated:

1. Accumulation of atmospheric 2-nitropropane (2-NP) in rats
2. Rates of metabolism of 2-NP in rats
3. Liver enzymes in blood serum of rats after single IP doses of 2-NP
4. Liver foci bioassay in rats after exposure to atmospheric 2-NP
5. Development of a software for a sophisticated pharmacokinetic analysis

Following publications have appeared or are in press:

- J.G. Filser and M. Baumann (1988) Pharmacokinetics of 2-nitropropane in rats and determination of enzymes in serum specific for liver injury. Naunyn-Schmiedeberg's Arch. Pharmacol., Suppl. 337, R 17.

- W. Kessler, B. Denk and J.G. Filser (1989) Species-specific inhalation pharmacokinetics of 2-nitropropane, methyl ethyl ketone, and n-hexane. in C.C. Travis (ed.) Biologically-based Methods for Cancer Risk Assessment, pp. 123-139, Plenum, New York.

- B. Denk, M. Baumann and J.G. Filser (1989) Pharmacokinetics and hepatotoxicity of 2-nitropropane in rats. Arch. Toxicol., Suppl. 13, 330-332.

- D. Henschler (ed.) (1989) Deutsche Forschungsgemeinschaft "Gesundheitsschädliche Arbeitsstoffe", Toxikologisch-arbeitsmedizinische Begründung von MAK-Werten, 2-Nitropropan, Nachtrag 1989, 15. Lieferung, Verlag Chemie, Weinheim

- B. Denk, J.G. Filser, E. Deml, W. Kessler, J. Shen and D. Oesterle (1990) Dose-dependent emergence of preneoplastic foci in rat livers after exposure to 2-nitropropane. Arch Toxicol., in press.

METHODS

If not mentioned otherwise methods used have been described in the proposal.

Kinetic calculations:

Pharmacokinetic parameters of 2-NP were obtained from exposures of male and female Sprague-Dawley rats (200- 250 g) to initial atmospheric concentrations (100 - 3000 ppm) in closed chambers, occupied by 2 animals each. The concentration-time courses were analyzed by means of the two compartment model shown in Fig. 1. In this model the atmosphere of the closed chamber is considered to be the first and the whole organism of the exposed animals the second compartment. The processes of inhalative uptake and of exhalative elimination are treated linearily, i.e., the rates of inhalation and exhalation are directly proportional to the actual concentration in the atmosphere and the actual average concentration in the organism, respectively. The elimination constant k*el could be described as a function of the concentration and was composed of two different metabolic processes: a non-saturable one following first-order kinetics and a saturable one. Using this model, concentration-time curves were iteratively calculated with a personal computer and fitted through the measured values. With the obtained parameters a conversion for an open system with an infinitely large atmosphere was carried out.

Liver foci bioassay:

Male and female Sprague-Dawley rats, 4-6 days old, were exposed together with their dams for 3 weeks (6 h/d, 5 d/w) to 2-NP vapours of 0, 24±1.3, 40±4.9, 50±2.7, 80±5.6, 123±5.0, and 200 ppm. Animals at this age were used because younger rats showed a higher sensitivity in the foci bioassay. During the exposure period animals were housed in open inhalation chambers (240 l, flow of prefiltered air of 25°C, 80 l/min). One week later polychlorinated biphenyls (Clophen A50, 10 mg/kg) were applied for promotion twice a week for 8 weeks. One week later all animals were killed under ether anesthesia. From each animal, two liver lobes were removed and frozen. Cryostat sections were prepared and stained for the demonstration of the number and area of preneoplastic liver foci deficient in adenosine-5'-triphosphatase.

RESULTS

1. Accumulation of atmospheric 2-NP

The enrichment of 2-NP was determined in water and olive oil. The resulting Ostwald's partition coefficients of 2-NP were 128 for water/air and 710 for olive oil/air at 37°C. From these values the thermodynamic partition coefficient (body/air) for rats was estimated to be 161, assuming that the body of a rat consists of 70 % aqueous and 10 % fatty compartments. A similar thermodynamic partition coefficient of 180 was obtained in both sexes from the pharmacokinetic analysis of the semilogarithmic concentration-time courses of 2-NP in the atmosphere of the exposure chamber (Fig. 2a and b; Table 1). However, the accumulation of the substance cannot reach this theoretical value because of its metabolism. A concentration ratio in steady state (body/air) was calculated (Table 1) which gives the accumulation at certain atmospheric concentrations (Fig. 3). It declines to 23 and 30 in male and female rats, respectively, at concentrations below 10 ppm. The clearance of uptake was remarkably high, even higher than the ventilation rate of the rat. This may be caused by a remarkable skin uptake. Only small amounts of unchanged 2-NP were exhaled as calculated from the ratio of the clearance of exhalation to the clearance of uptake: 13 % in males and 18 % in females.

2. Metabolism of 2-NP

The best fits of the pharmacokinetic analysis of the concentration-time courses (Fig. 2a and b) served to obtain the pharmacokinetic parameters listed in Table 1. To obtain this goodness of fit k*el had to be expressed as a function composed of two different metabolic pathways in both sexes:
a non-saturable metabolism according to first-order kinetics with a kel which was quite similar in both sexes and
a saturable metabolism according to Michealis-Menten kinetics with low capacity and high affinity which revealed striking sex differences (Table 1).
In females $Km_{app.}$ (related to the concentration in the body) was 3.2 times and Vmax 2.3 times higher than in males. At steady-state, the atmospheric concentration of 2-NP at Vmax/2 was 71 ppm in females and 28 ppm in males.
Calculated rates of the different metabolic processes in males and females are given in Fig. 4. Since 2-NP is metabolized partly by the saturable pathway, the solid lines demonstrating the non-saturable pathways are curved. In females more 2-NP is metabolized by the non-saturable than by the saturable pathway at concentrations above 180 ppm, in males already at concentrations above 60 ppm.
In order to inhibit cytochrome P450 dependent metabolism experiments were performed with rats which were pretreated by intraperitoneal injection of diethyldithiocarb, 200 mg/kg. Immediately after starting the exposures the slopes of the concentration-time courses decreased compared to pretreated controls (Fig. 5) resulting in an inhibition of 2-NP uptake by 51 %. The most likely explanation for these findings is a

dramatic reduction in breathing rate due to combination effect of 2-NP with dithiocarb. These data have therefore not been used for kinetic analysis.

The possible occurence of a first pass effect due to metabolism in the liver was investigated by administering 2-NP intraperitoneally to female and male rats at doses of 1.7 mmol/kg (males and females) and 0.17 mmol/kg (females). Immediately thereafter, the animals were placed in the closed chambers and the concentration-time courses of 2-NP in the atmosphere of the chambers were measured (experimental data will be shown in the final report). Comparison of the measured data with curves predicted from the inhalation studies by means of the two-compartment model yielded first pass effects of 23 % in females (both doses) and of 15 % in males.

3. Liver enzymes in blood serum after single IP doses of 2-NP

2-NP, 1.7 mmol/kg b.w., was administered IP to female and male rats. After eight hours the liver enzymes glutamic oxalacetic transaminase (GOT), glutamic pyruvic transaminase (GPT) and ornithine carbamyl transferase (OCT), used as markers for acute liver damage, reached peak concentrations in blood serum (Fig. 6). The values in females were 1.9-fold (GOT), 2.7-fold (GPT), and 7.9 (OCT) higher than in controls. In males the corresponding values were 22-fold (GOT), 20-fold (GPT), and 460-fold (OCT) higher than in controls. The dose-response relationship of IP administrated 2-NP and liver enzymes was investigated in male rats at

concentrations from 0.13 to 3.4 mmol/kg. Typical dose-response curves were found using the enzyme activities as parameters (Fig. 7). In every case the maximum effect was reached after dosing 1.7 mmol/kg 2-NP.

4. Liver foci bioassay after exposure to atmospheric 2-NP

Exposure to 200 ppm 2-NP had to be discontinued due to a high mortality of the pups within a few days. At the lower exposure concentrations animals did not show any signs of sickness. Body weight gain was not impaired; liver weight was slightly enhanced as compared to controls.
The exposure of rats to 2-NP with subsequent treatment with Clophen A50 induced ATPase-deficient preneoplastic foci in a dose-dependent manner (Fig. 8a and b). The resulting curves showed an upwards concave shape, indicating that at low concentrations the effect is relatively less pronounced than at high concentrations (Fig. 8a). Control rats with promotion only showed a foci number of 0.19/cm^2 of liver section for male and 0.73/cm^2 for females. Male rats exhibited a significant, about fourfold lower foci incidence than females throughout the concentration range between 0 and 125 ppm of 2-NP. The number of foci/cm2 was significantly different from controls at all concentrations in females, and at concentrations higher than 40 ppm in males. In the semilogarithmic plot linear dose-effect curves were observed with the same slope for both sexes (Fig. 8b). The lower intercept of the curve got with the data from males is most likely caused by a lesser susceptibility of male rats towards the promoting stimulus of Clophen A50 (Deml and Oesterle 1982).

5. Development of a program for a sophisticated pharmacokinetic analysis

A program "SOLVEKIN" was developed which is able to solve special and toxicokinetic problems as simulation and parameter estimation for data representing single curves or a set of curves . These curves can be described by functions or by first order differential equations.
SOLVEKIN is based on the simplex algorithm of Nelder and Mead (1965). The first order differential equations are solved with different methods (Runge-Kutta, Adams-Moulton, Bulirsh-Stoer) with error estimation and automatic stepsize control. The advantage of this procedure compared to similar programs (e.g. SIMUSOLV) is that sets of curves are treated as a surface and are fitted simultaneously. The program was written in C, because it allows a dynamic programming style and makes porting easy. It can be compiled without further changes in the source under VMS, UNIX, MSDOS, MAC OS, and MIPS OS.
The program will be used for the proposed pharmacokinetic modelling.

PRELIMINARY CONCLUSIONS

Male rats showed a higher susceptibility in the hepatotoxicity (Lewis et al. 1979), hepatocarcinogenicity (Griffin et al. 1980) and genotoxicity (Andrae et al. 1988) of 2-NP than female rats. These sex differences cannot be attributed to the substance itself, because the enrichment of atmospheric 2-NP in the body at steady-state conditions is higher in female than in male rats. They rather correlate with the share of the non-saturable metabolism found from the pharmacokinetic studies which is comparatively higher in males than in females.

The unusual shape of the concentration-time course of the liver foci bioassay also can be explained by the share of the non-saturable metabolism demonstrated in Fig. 5. The identical slopes of the foci incidence-concentration curves suggest an identical initiating efficiency of 2-NP in sucklings of both sexes.

From the two pathways, the saturable one, also observed in male rats by Nolan et al. (1982), is more likely to lead to less toxic or carcinogenic metabolites since at the same concentration of 2-NP this pathway is slower in male than in female adult rats. In contrast, the non-saturable pathway is assumed to be responsable for more potent toxic and carcinogenic metabolites which have not yet been identified. Perhaps the shift of 2-NP to its nitronate anion which can occur under physiological conditions (Fiala et al. (1987) may represent the non-saturable metabolism. This anion can further be oxidized by peroxidases to a free radical which is able to oxidize DNA directly or indirectly via oxygen radicals (Fiala et al. 1989).

WORK IN PROGRESS

We abolished the experiments with mice which were emphasized in the proposal. Instead of this, we are currently investigating the inhalation pharmacokinetics of 2-NP in male and female rabbits which, like rats, have been used in a long-term carcinogenicity study. In contrast to rats, rabbits showed no tumors up to exposure concentrations of 200 ppm of atmospheric 2-NP (Lewis et al. 1979). This difference may perhaps be explained by the shares of the different metabolic pathways in both species and sexes.

The liver foci bioassay was performed with suckling rats because of their higher susceptibility to initiating agents (Oesterle and Deml 1983). In a second liver foci bioassay adult female and male rats will be used to study a possible sex difference of the initiating potency of 2-NP.

In order to extrapolate the findings concerning the metabolism of 2-NP in the liver to humans, we are also investigating the metabolism of 2-NP in liver microsomes of rats, rabbits and humans.

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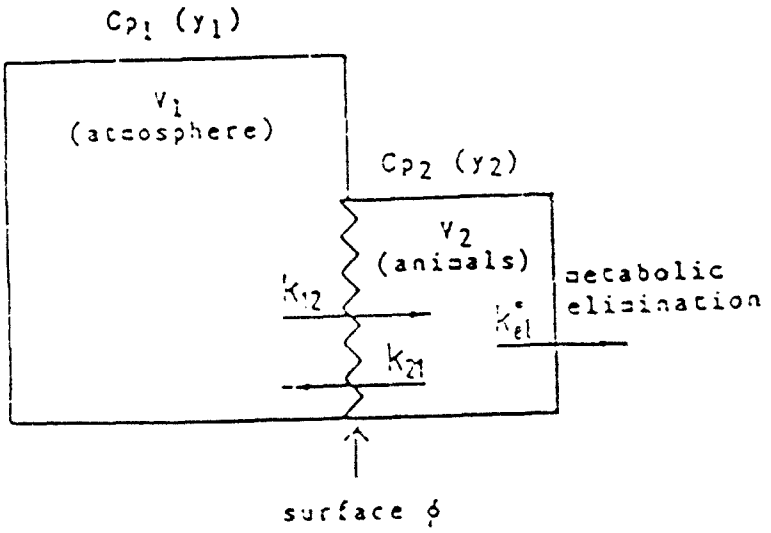
Table 1. Sex-Specific Pharmacokinetic Parameters of 2-NP in SD Rats (250 g)

Parameter	Value (female rat)	Value (male rat)	Dimension
Thermodynamic partition coefficient (body/air)	180	180	nl gas/ml tissue ppm in atmosphere
Concentration ratio in steady state (body/air)[1]	30	23	nl gas/ml tissue ppm in atmosphere
Clearance of uptake (related to atmosph.conc.)	180	180	ml/min
Clearance of exhalation[1] (related to atmosph.conc.)	32	24	ml/min
Clearance of metabolism[1] (related to atmosph.conc.)	150	160	ml/min
Clearance of non-saturable metabolic pathway[1](related to atmosph.conc.)	25	32	ml/min
Vmax	0.48	0.21	µmol/min
Kmapp.	2900	900	nl gas/ml tissue

1: valid for atmospheric concentrations less than 10 ppm

Fig. 1 Toxicokinetic two-compartment model (first-order-kinetics for inhalation and exhalation; first-order-kinetics and Michaelis-Menten-kinetics rsp. for metabolism)



Cp_1: Compartment 1. Cp_2: Compartment 2

y_1 : Concentration in the atmosphere. y_2 : Concentration in the organism

V_1 : Volume of the atmosphere. V_2 : Volume of the animals

k_{12}: Microconstant of the uptake process

k_{21}: Microconstant of the exhalation process

k^*_{el}: Microconstant of the metabolic elimination (concentration dependent)

$$k^*_{el} = \frac{V_{max}}{V_2 \cdot (K_{m(app)} + y_2)} + k_{el}$$

V_{max} : Maximal rate of saturable pathway

$K_{m(app)}$: Apparent Michaelis-Menten constant of saturable pathway

k_{el} : Microconstant for non-saturable pathway

Differential equations related to the pharmakokinetic model:

atmosphere: $V_1 \cdot \frac{dy_1}{dt} = -k_{12} \cdot V_1 \cdot y_1 + k_{21} \cdot V_2 \cdot y_2$

animal: $V_2 \cdot \frac{dy_2}{dt} = k_{12} \cdot V_1 \cdot y_1 - (k^*_{el} + k_{21}) \cdot V_2 \cdot y_2$

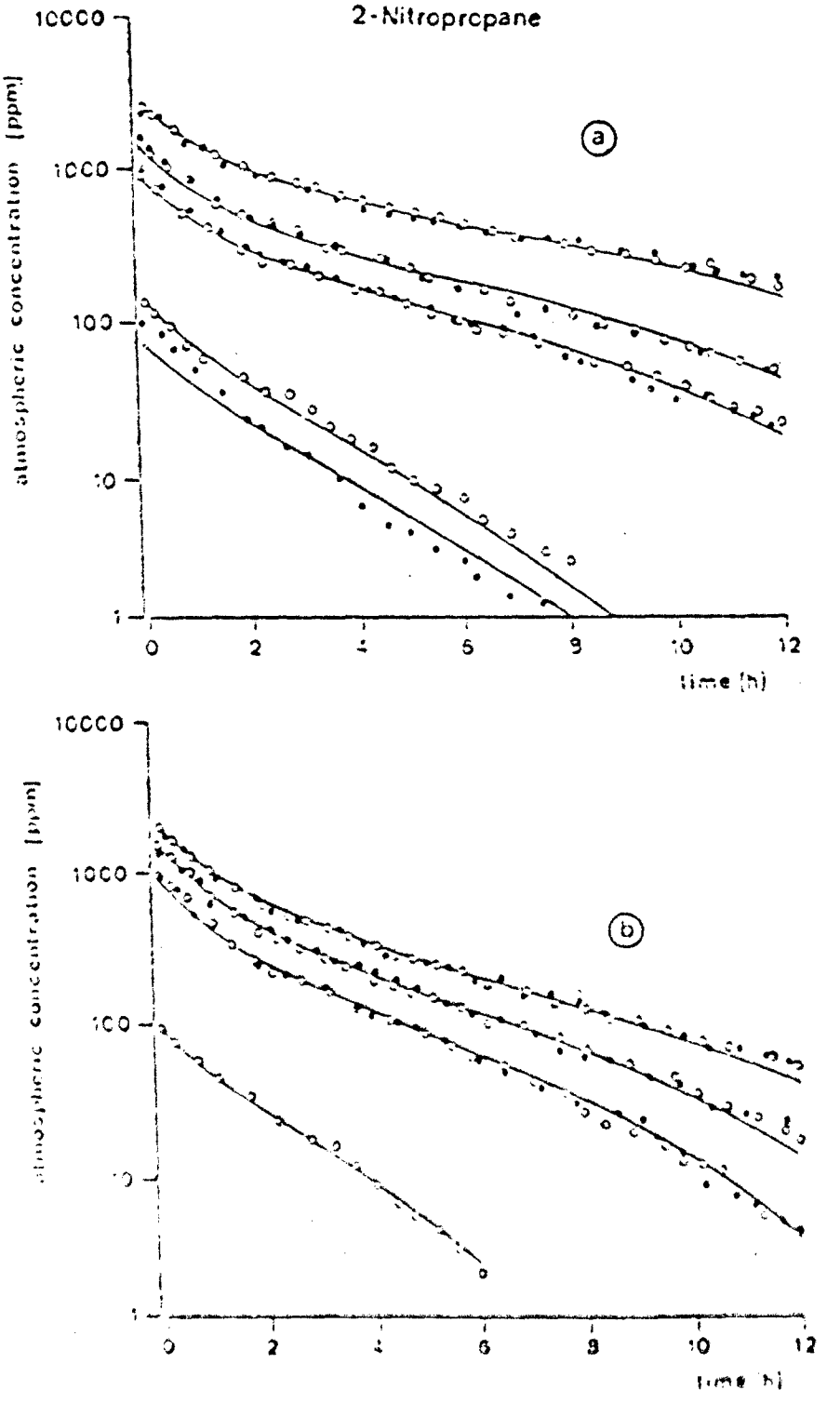


Fig. 2. Concentration-time curves of inhaled 2-NP at different initial concentrations in the gas phase of a closed exposure chamber occupied by two 20 rats.
(a) females, (b) males
Dots: measured values; lines: calculated curves using the kinetic data given in table 1.

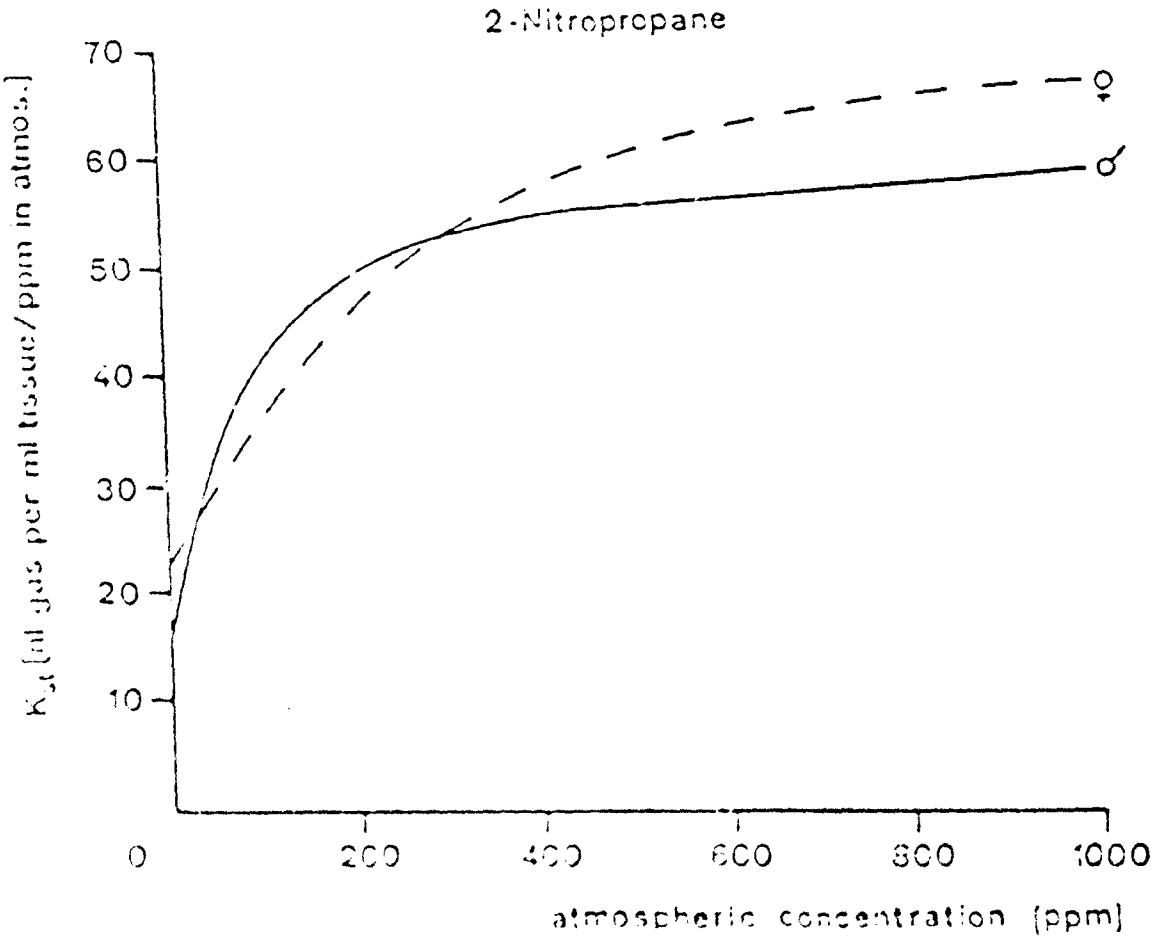


Fig. 3 Enrichment of 2-NP in the rat organism at steady state in dependence of its concentration in the atmosphere
Dashed lines: female rats; solid lines: male rats; K_{st}: Steady-state-constant calculated for 1 kg b.w. (2 rats of 500 g each)

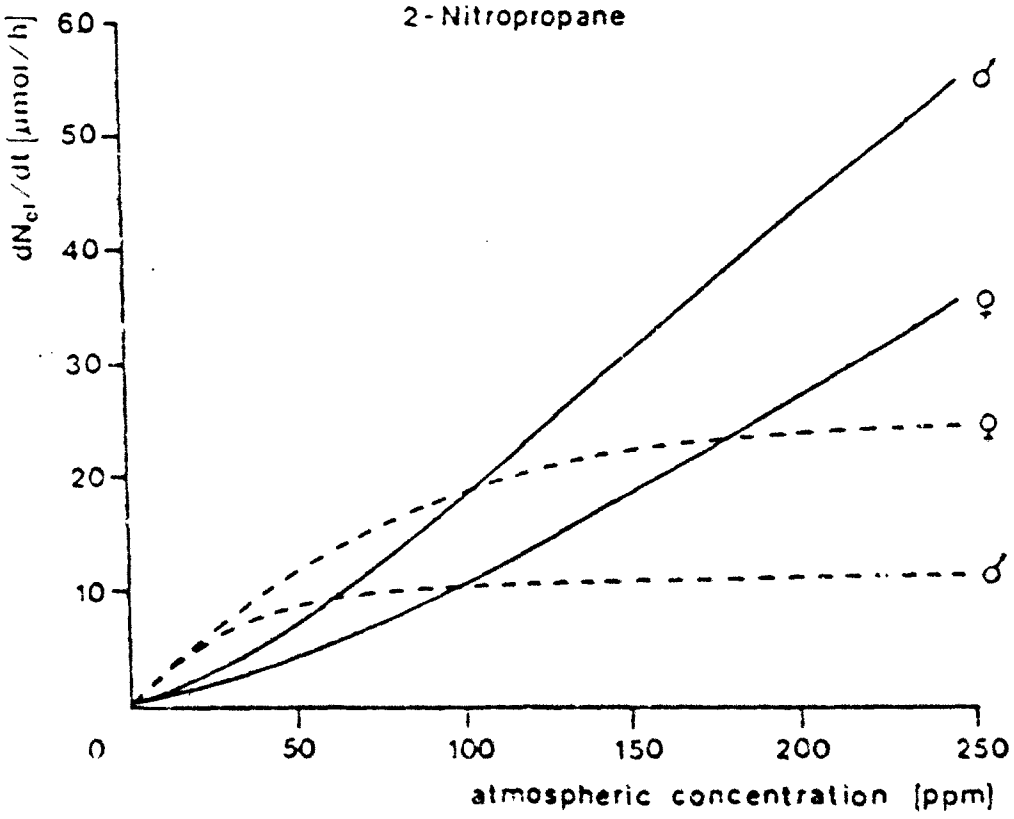
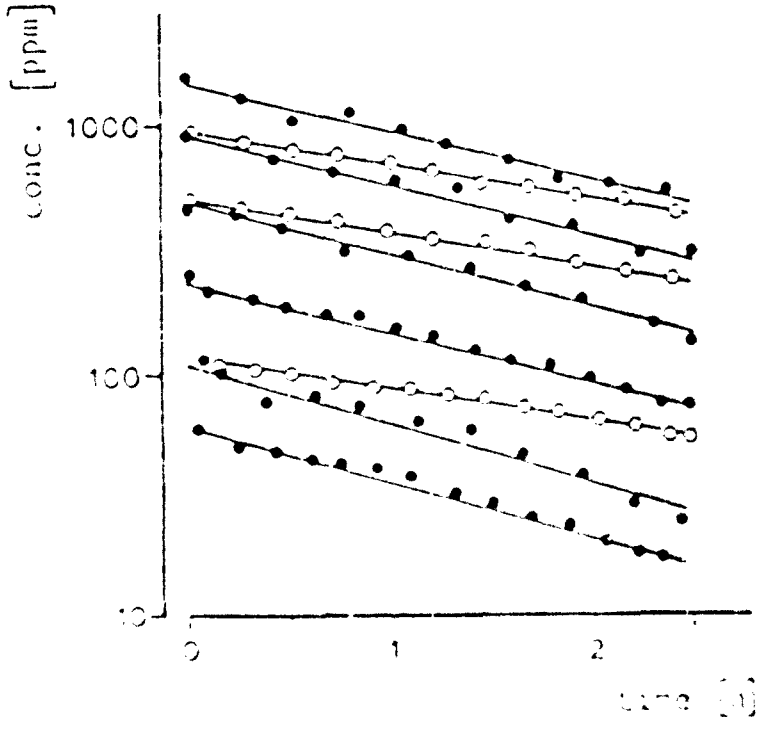


Fig. 4. Rate of metabolism (dNel/dt) at steady state of 2-NP in a SD rat of 250 g dependent on the atmospheric concentration, calculated for an open exposure system. Dashed lines: saturable metabolic pathway; solid lines: non-saturable metabolic pathway

Fig. 5: Concentration-time curves of 2-nitropropane in the atmosphere of the exposure system, occupied by two rats



data measured, linear calculated
●—● separately ●—○ pretreated with disulfiram

GOT AND GPT

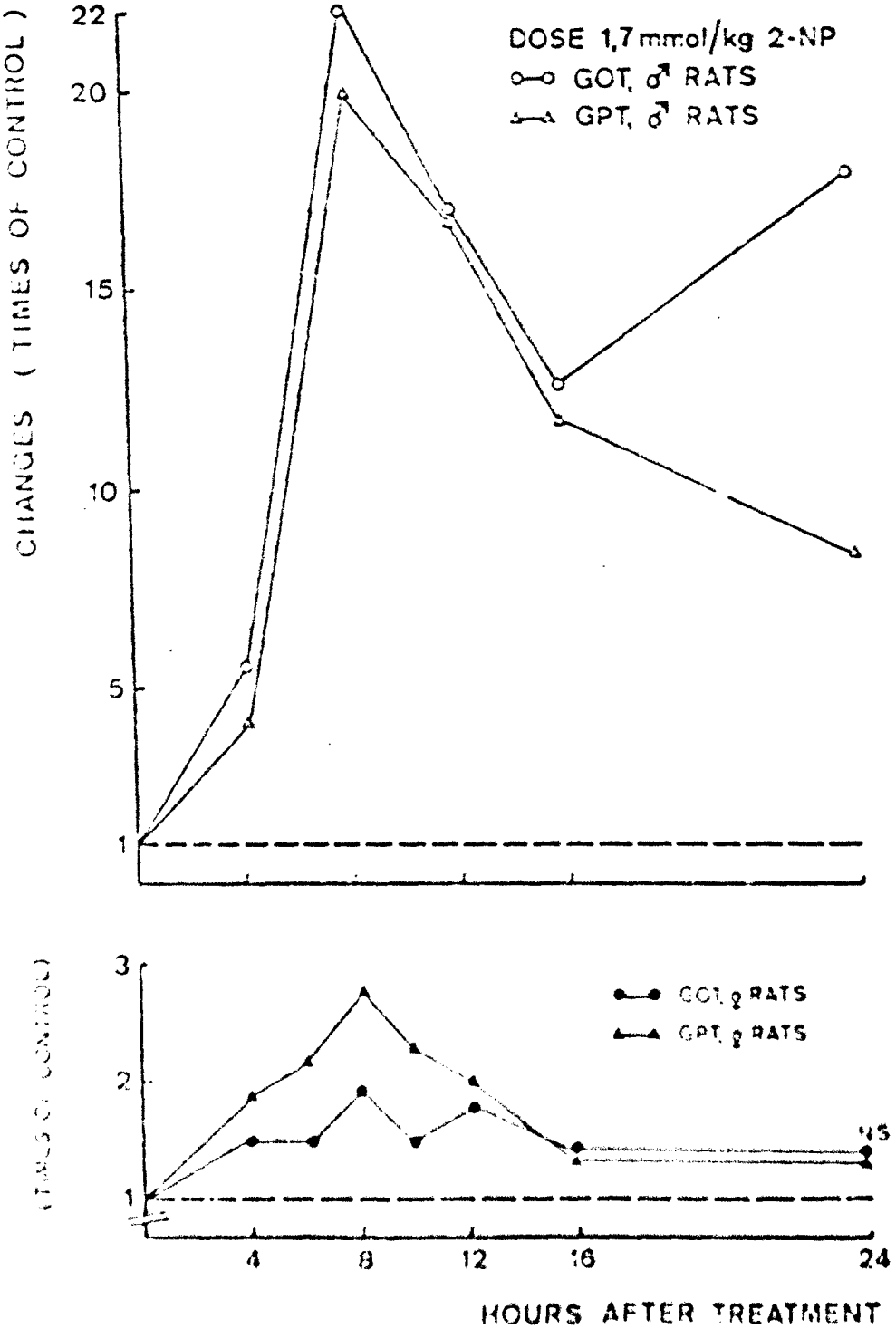


Fig.6 Time-course of the activities of GOT and GPT in serum of female and male rats after single IP-doses of 2-NP (1.7 mmol/kg b.w.t.). Data are given as multiple changes of controls. NS: not significant (n=6)

DOSE-RESPONSE-CURVES

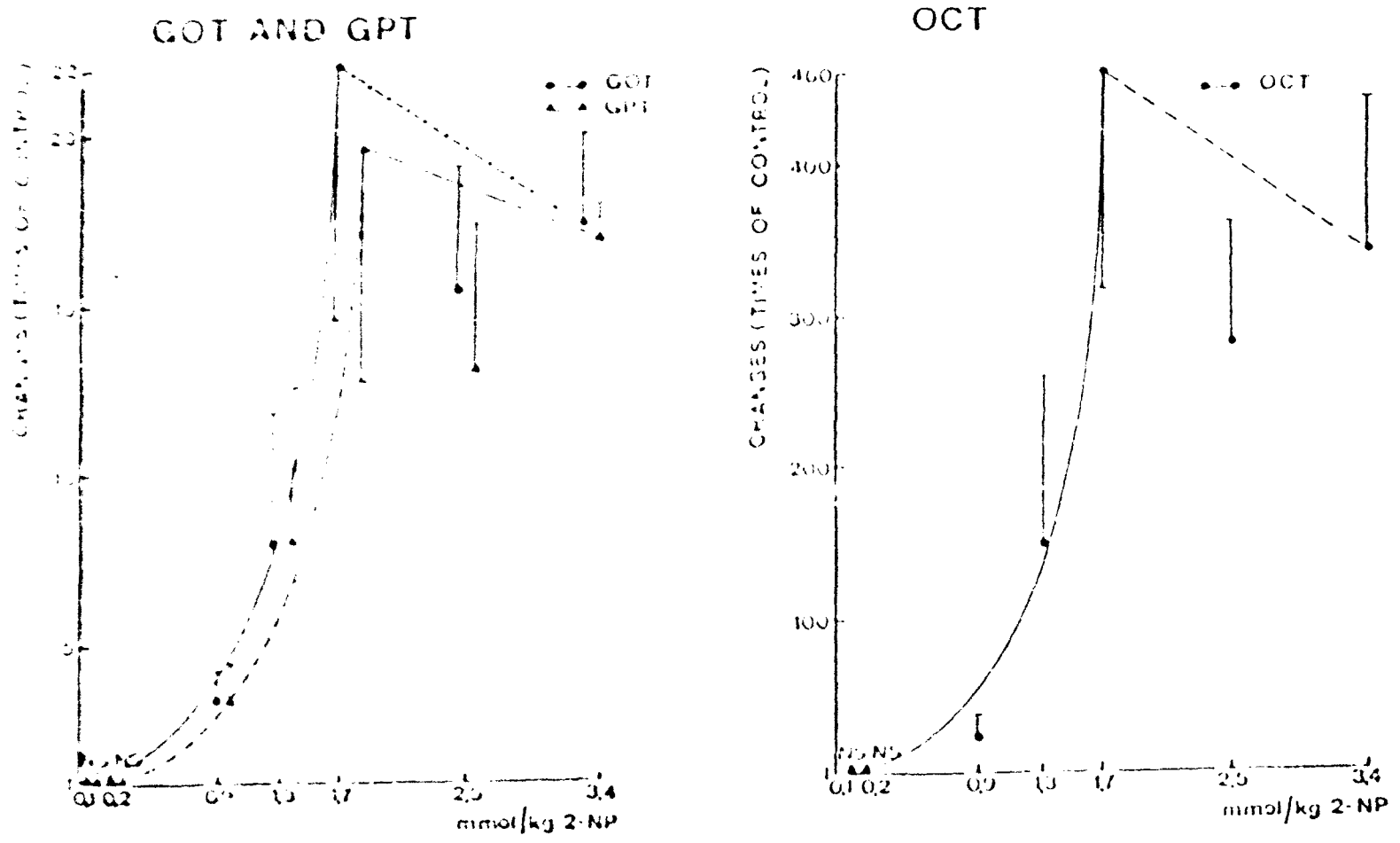
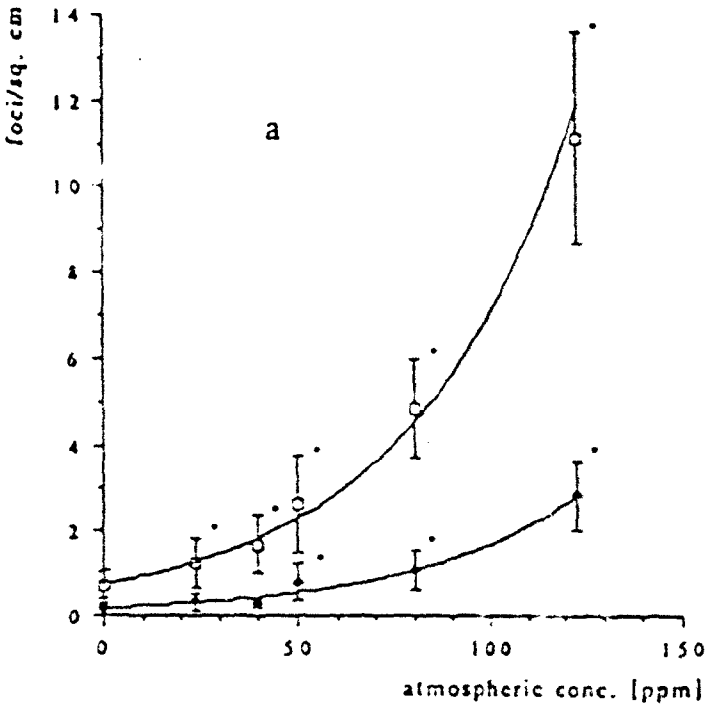


Fig. 7 Activities of GOT, GPT, and OCT in serum of male rats 8 hours after single IP-injections of 2-NP (doses from 0.13 to 3.4 mmol/kg b.wt). Data are given as multiple changes of controls. NS: not significant (n=6)



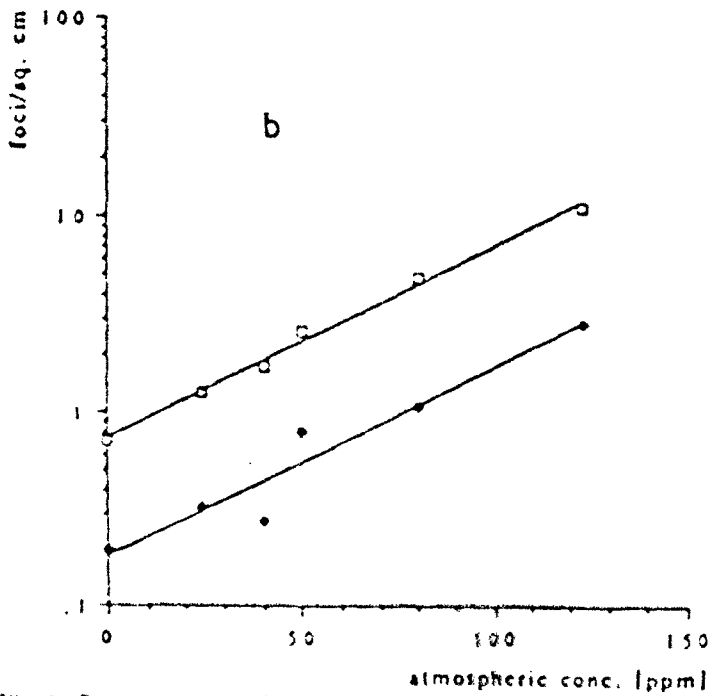


Fig. 8. Preneoplastic ATPase-deficient foci in the livers of rats after exposure to 2-NP. a Linear graph [means ± standard deviations (controls: $n = 12$; exposed animals: $n = 6$)]. *Upper curve:* females ($y = 0.75 \cdot 10^{0.027x}$; $r = 0.996$); *lower curve:* males ($y = 0.15 \cdot 10^{0.020x}$; $r = 0.963$). * significantly different from controls: $2p < 0.05$. b Semilogarithmic graph (means). *Upper curve:* females ($y = 0.75 \cdot 10^{0.027x}$; $r = 0.996$); *lower curve:* males ($y = 0.15 \cdot 10^{0.020x}$; $r = 0.963$). For details see text

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